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DEVELOPMENT AND EXPERIMENTAL CALIBRATION OF THE MATHEMATICAL MODEL OF LIPID PEROXIDATION IN MOUSE LIVER SLICES

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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FOR THE DIRECTOR

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13. ABSTRACT (Maximum 200 words)

A computerized biologically based pharmacodynamic (BBPD) model was developed in order to describe and simulate chemically induced lipid peroxidation in precision cut mouse liver slices. The model was written in Advanced Continuous Simulation Language (ACSL) and simulations were performed using SIMUSOLV software with optimization capabilities on a VAX/VMS mainframe computer. The BBPD model simulated formation of lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) over time as a function of the amounts of cytochrome P450 (CYP)-activated chemical inducer and active antioxidants. The rate of peroxidation was controlled by lipid peroxidizability, destruction of CYP, autooxidation, and activity of glutathione peroxidase. The BBPD model was initially parameterized with the literature data for TBARS formation during lipid peroxidation, reported for rat liver slices induced with bromotrichloromethane and tert-butyl hydroperoxide (TBOOH). Then, the biochemical parameters were adjusted to reflect the physiology of the mouse liver, and the BBPD model was used to simulate TBARS formation during lipid peroxidation in precision cut mouse liver slices induced with TBOOH. The BBPD model predictions were in agreement with the experimental data.

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PREFACE

This report describes the results of the development and experimental validation of a mathematical model simulating chemically induced lipid peroxidation in precision cut mouse liver slices. This is one of a series of technical reports and publications describing results of a collaborative effort conducted by ManTech Environmental Technology, Inc., Toxic Hazards Research Unit, located at Wright-Patterson Air Force Base, and by Occupational and Environmental Health Directorate, Toxicology Division, and aimed at pharmacodynamic description of biological effects of chemical compounds.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

Research performed by ManTech Environmental was conducted under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F39) and research by the Toxicology Division was conducted under AFOSR Work Unit No. 2312A202. Funding was provided to the Toxicology Division by the AFOSR Basic Environmental Initiative Project.

Lt Col Terry A. Childress, Director of the Toxicology Division, served as Contract Technical Monitor.

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ABBREVIATIONS

ACSL   Advanced Continuous Simulation Language

BBPD   Biologically-based pharmacodynamic

CYP   Cytochrome P450

g   Gram

GSH   Glutathione reduced

HPUF   Heavily polyunsaturated fat

L   Liter

LDH   Lactate dehydrogenase

LPUF   Lightly polyunsaturated fat

MDA   Malondialdehyde

PBPK   Physiologically based pharmacokinetic

TBA   Thiobarbituric acid

TBARS   Thiobarbituric acid reactive substances

TBOOH   Tert-butyl hydroperoxide

Symbols used in the model are listed in the legend to Figure 1.

SECTION 1

INTRODUCTION

Lipid peroxidation is a process involving avalanche-type chain reactions occurring in living cells that may be initiated by free radicals generated by both normal metabolic pathways and by oxidative stress caused, for instance, by metabolic activation of xenobiotics. Essentially, the process incorporates molecular oxygen into polyunsaturated fatty acids (PUFA) to yield hydroperoxides as the primary initial product, and which may be destructive to phospholipid biomembranes in the tissues. This process, in the course of its initiation, propagation, and termination steps, yields several types of secondary free radicals (peroxyl, hydroperoxyl, alkoxyl) and several non-radical products (aldehydes, alkanes, alkenes, ketones, and hydroxy acids; Gardner, 1989) which may further interact with proteins and other cellular macromolecules (Allevi et al., 1995).

Lipid peroxidation was implied as one of the mechanisms involved in, or as a biomarker indicative of tissue injury by several xenobiotics (Kappus and Sies, 1981), by radiation (Slater, 1984), by hypoxia (De Groot and Littauer, 1989) and by inflammation (Flohe et al., 1985). Lipid peroxidation also plays a role in genotoxicity (Brambilla et al., 1989; Chaudhary et al., 1994), in disruption of signal transmission (Van Der Vliet and Bast, 1992), and in tumor promotion (Byczkowski and Channel, 1995; Perchellet and Perchellet, 1989). Accordingly, it was demonstrated that the tumor-promoting tert-butyl hydroperoxide (TBOOH) affected the activity of protein kinase C (by a mechanism involving lipid peroxidation in hepatocytes; (Von Ruecker et al., 1989). Protein kinase C takes part in the transduction of cellular proliferative signals via growth factors (Nicotera et al., 1992).

An increased understanding of the causative role of lipid peroxidation in toxicology prompted us to explore the possibility of quantitative modeling of this complicated multistep process. Existing mathematical models of lipid peroxidation (Tappel et al., 1989; Babbs and Steiner, 1990) were unsuitable for interlinking with physiologically based pharmacokinetic (PBPK) models used to simulate tissue distribution of toxicants in vivo (Yang and Andersen, 1994). As described in this report, we have developed a computerized biologically based pharmacodynamic (BBPD) model which simulated chemically induced lipid peroxidation in precision cut mouse liver slices, based on the mathematical description by Tappel et al. (1989). This PBPK-compatible model was experimentally verified with data for lipid peroxidation initiated by TBOOH (Byczkowski et al., 1995).

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SECTION 2

MATERIALS AND METHODS

The model was written in Advanced Continuous Simulation Language (ACSL; Mitchell and Gauthier Associates, 1993), and simulations were performed using SIMUSOLV software with optimization capabilities (DOW Chemical Co., Midland, MI) on a VAX/VMS mainframe computer.

Precision cut liver slices were prepared from B6C3F1 male mice (Charles River Breeding Laboratories, Kingston, NY) and maintained using the dynamic roller culture method (Sipes et al., 1987; Brendel et al., 1993). The mice were provided with Purina Forumlab #5008 and softened water *ad libitum*.

The mice were euthanized with CO_2 and their livers were removed and placed in ice-cold Sacks buffer (containing: KH_2PO_4 0.75 g/L, K_2HPO_4 9.5 g/L, $NaHCO_3$ 1.2 g/L, $KHCO_3$ 0.6 g/L, mannitol 37.5 g/L and $MgCl_2$; pH 7.4). Eight mm liver cores were prepared and sliced in ice-cold Sacks buffer using a Krumdieck tissue slicer (Alabama Research and Development, Munford, AL; Brendel, 1987; Krumdieck, 1980). The slices were loaded on rollers (two slices per roller) in ice-cold Sacks buffer. The rollers were then placed in scintillation vials containing 1.7 mL of Waymouths MB 752/1 media at 37 ^{o}C (Formula # 78-5107EC, without phenol red, pH 7.4, Gibco BRL, Grand Island, NY), supplemented with: $NaHCO_3$ 1.3 g/L, HEPES 2.38 g/L, NaCl 0.292 g/L, l-glutamine 0.35 g/L, gentamycin sulfate 50 mg/L, and 10% fetal bovine serum (Hyclone, Logan, UT) and capped with a scintillation vial cap with 1/4" hole for gas exchange. The vials were placed in a Dynamic Roller Culture Incubator (Vitron, Tucson, AZ) and gassed with 95% O_2/5% CO_2 for a 2-hour preincubation period.

After a 2-hour preincubation period, the rollers were removed from the vials and placed into prewarmed vials containing the media (pH 7.4) dosed with TBOOH at the desired final concentration. The dosed vials containing rollers were then returned to the roller culture incubator. Zero time controls were processed immediately. Then, the vials were removed at 20-minute intervals over a 2-hour incubation. Slices were weighed and sonicated in their own media. Aliquots of each sample were removed for thiobarbituric acid reactive substances (TBARS) assay and protein content measurements. Samples for TBARS assay were added to ice-cold D-PBS/GSH/EDTA buffer (pH 7.4) containing: 20 mg reduced GSH and 48 mg EDTA in 100 mL D-PBS (Dulbeco's buffer; Gibco BRL, Grand Island, NY).

Lipid peroxidation was measured by the formation of TBARS, employing the fluorescence spectrophotometry of solvent tissue extracts (Janero, 1990). Essentially, in this assay the aldehyde products generated by splitting the endoperoxide alkoxyl radicals (mostly malondialdehyde, MDA, formed during the peroxidation of unsaturated fatty acids) react with thiobarbituric acid (TBA) to yield a 1:2 MDA:TBA red, fluorescent, complex (Janero, 1990). Under conditions of the assay, the amounts of MDA:TBA complex, and thus color and fluorescence intensity, are stoichiometrically depending on lipid hydroperoxides and rate of their production corresponds to the rate of lipid peroxidation.

At 1 hour and 2 hours of incubation, the samples of liver slices (control and treated) were removed for viability analysis. The extent of cytotoxicity was estimated from lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) leakage; and intracellular potassium content. The enzyme leakage was determined using a Kodak Ektachem Analyzer (model 700XR) for aminotransferase activities and DuPont acaV for the dehydrogenase activity. The acceptable enzyme leakage level for precision cut liver slices was assumed to be less than 20% of the total content of enzymatic activity. Potassium content in sonicated tissue samples was determined using an AVL 982-S Electrolyte Analyzer (Roswell, GA). The acceptable level of intracellular potassium content in precision cut liver slices was assumed to be greater than 35 mM K^+/g wet weight. If the average viability tests of liver samples did not meet the above acceptable levels, the experimental results were discarded.

All chemicals used in this study were of analytical grade.

SECTION 3

RESULTS

BBPD Model Structure

A general simplified scheme of the BBPD model is shown in Figure 1. The model was based on a mathematical description of lipid peroxidation by Tappel et al. (1989), who developed a spreadsheet routine for iterative calculations using LOTUS 123 software. We used their terminology, whereas their symbols were modified to meet requirements of the ACSL programming language.

The model described generation of the peroxides and, finally, TBARS from polyunsaturated fat during lipid peroxidation (the reactants are depicted by rectangles in Figure 1) which were increased by stimulators (the stimulators are depicted by ovals in Figure 1) and decreased by inhibitors (the inhibitors are depicted by triangles in Figure 1). The amounts were expressed in micromoles per 0.1 g of liver. The mass transfer between different pools of reactants was modified by conversion factors expressed either as yields (micromole per micromole) or rates (micromole per hour).

The BBPD model simulated formation of lipid hydroperoxides and TBARS over time as a function of remaining amounts of CYP-activated chemical inducer and remaining amounts of antioxidant. The rate of peroxidation was controlled by lipid peroxidizability, destruction of CYP, autooxidation, and the activity of glutathione peroxidase.

The process was initiated when lightly and heavily polyunsaturated fat (LPUF, HPUF) reacted with activated inducer (ACTIND) that abstracts hydrogen. The "activated inducer" depicts a free radical reactive intermediate produced from xenobiotic by CYP (tissue activator). The CYP may be partly destroyed during this reaction, and this amount was subtracted from the pool of effective activator. The amount of inducer that already abstracted hydrogen (INDLOS) was also subtracted from the pool of effective inducer. The amounts in the pool of peroxidized fat (PXLUFA and PXHUFA) were controlled by fatty acids peroxidizability and peroxidation rate (PXRATE) of PUF, as well as by the amount of antioxidants (ANOXRE). Additional amounts of peroxidized fat were produced by autooxidation (AUTOXA). The process of autooxidation was initiated by accumulated total hydroperoxides (TPX) and was controlled by the autooxidation factor (AUTOXF rate).

Figure 1. A general simplified scheme of the BBPD model for simulation of lipid peroxidation in precision cut liver slices in vitro. Description of symbols: PUF - polyunsaturated fat; LPUF - lightly polyunsaturated fat; HPUF - heavily polyunsaturated fat; PXLUFA - peroxidized lightly polyunsaturated fat; PXHUFA - peroxidized heavily polyunsaturated fat; PXRATE - peroxidation rate; TPX - accumulated total hydroperoxides; AUTOXA - hydroperoxides produced by autooxidation; AUTOXF - autooxidation factor rate; PXREM - accumulated remaining hydroperoxides; TBARS - thiobarbituric acid reactive substances; PXTTBA - yield of TBARS from hydroperoxides; PXREDA - reduced hydroperoxides; PXREDF - hydroperoxide reduction factor rate; IND1 - dose of chemical inducer 1; ACTIND - activated chemical inducer; INDLOS - inactive inducer; ANOX1 - vitamin E-type antioxidants; ANOXRE - remaining antioxidants; ANOXLOS - inactive antioxidants; ANOXUF - antioxidant use factor; GSH - amount of reduced glutathione; GSHREM - remaining reduced glutathione; GSSG - used glutathione.

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The accumulated total hydroperoxides may be partly reduced by glutathione peroxidase in the reaction controlled by hydroperoxide reduction factor (PXREDF rate) and the amount of reduced glutathione (GSH). The amount of used glutathione (GSSG) equivalent to the amount of produced hydroxy fatty acids (PXREDA) was subtracted from the pool of reduced glutathione. The amount of produced hydroxy fatty acids was also subtracted from the pool of total hydroperoxides, leaving the amount of accumulated remaining hydroperoxides (PXREM) representing an end product of the peroxidative degradation of PUF.

The pool of accumulated remaining hydroperoxides was subjected to further free radical reactions, yielding the thiobarbituric acid reactive substances (TBARS). The yield of TBARS (in micromole of TBARS per micromole of hydroperoxide) was reflected in the coefficient PXTTBA.

The quantitative relations and governing equations, based on Tappel et al. (1989), are listed in Table 1, in the order in which they were called by the BBPD computer program.

The evolution of amounts of reactants over time was computed by numerical integration employing the Gear's algorithm for stiff systems (Mitchell and Gauthier, 1993). Initial physiological conditions, before lipid peroxidation, were set in the "INITIALIZATION" section, whereas safeguards preventing variables from assuming negative values and the amount of antioxidants to drop to zero, were set in the "PROCEDURAL" block of the BBPD program.

The source codes of *.CSL and *.CMD files are listed in the APPENDIX.

Table 1. Quantitative Relations and Governing Equations

Equation Number	Description
1)	Amounts of remaining polyunsaturated fatty acids = polyunsaturated fatty acids (PUFA) - (accumulated hydroperoxides formed by action of activated inducer on PUFA + accumulated hydroperoxides formed by autooxidation)
	[units: micromole/0.1 g liver]
2)	Amount of effective activator = ((concentration of activator 1) * (activity of activator 1)) + ((concentration of activator 2) * (activity of activator 2))
	[units: micromole/0.1 g liver]

Table 1. Quantitative Relations and Governing Equations (cont'd)

Equation Number	Description
3)	Activator loss = effective activator * activator degradation factor * total hydroperoxides formed . [units: micromole/0.1 g liver]
4)	Amount of remaining activator = effective activator - activator loss [micromole/0.1 g liver]
5)	Amount of effective inducer = ((concentration of inducer 1) * (potency of inducer 1)) + ((concentration of inducer 2) * (potency of inducer 2)) [units: micromole/0.1 g liver]
6)	Amount of remaining inducer = effective inducer - (effective inducer * reaction time * inducer loss factor) [units: micromole/0.1g liver]
7)	Inducer loss rate = effective inducer * inducer loss factor [units: micromole/0.1 g liver/hr]
8)	Amount of activated inducer = remaining inducer * remaining activator [units: micromole/0.1 g liver]
9)	Amount of effective antioxidant = ((concentration of antioxidant 1) * (effectiveness of antioxidant 1)) + ((concentration of antioxidant 2) * (effectiveness of antioxidant 2)) [units: micromole/0.1 g liver]
10)	Amount of remaining antioxidant = effective antioxidant - (effective antioxidant * total hydroperoxides formed * antioxidant use factor) + antioxidant regenerated [units: micromole/0.1 g liver]
11)	Rate of hydroperoxides formation by action of activated inducer on PUFA = (remaining polyunsaturated fatty acids * peroxidizability of polyunsaturated acids * activated inducer * peroxidation rate)/ remaining antioxidant [units: micromole/0.1 g liver/hr]
12)	Rate of autooxidation = (remaining PUFA * autooxidation factor * accumulated total hydroperoxides formed)/remaining antioxidant [units: micromole/0.1 g liver/hr]
13)	Amount of accumulated total hydroperoxides formed = (accumulated hydroperoxides from autooxidation of PUFA + accumulated hydroperoxides from action of activated inducers on PUFA) + physiological level [units: micromole/0.1 g liver]
14)	Amount of remaining glutathione = glutathione - accumulated reduced hydroperoxides [units: micromole/0.1 g liver]
15)	Rate of hydroperoxides reduced by glutathione peroxidase = total ydroperoxides * glutathione peroxidase * remaining glutathione * hydroperoxide reduction factor [units: micromole/0.1 g liver/hr]

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Table 1. Quantitative Relations and Governing Equations (cont'd)

Equation Number	Description
16)	Amount of accumulated remaining hydroperoxides = total accumulated hydroperoxides formed accumulated reduced hydroperoxides [units: micromole/0.1 g liver]
17)	Amount of TBARS from accumulated remaining hydroperoxides = accumulated remaining hydroperoxides * yield of TBARS from hydroperoxides + analytical blank [units: micromole/0.1 g liver]
18)	Inducer loss = integral of inducer loss rate [units: micromole/0.1 g liver]
19)	Amount of accumulated hydroperoxides formed by action of activated inducer on PUFA = integral of (accumulated hydroperoxides formed + hydroperoxides formed) over time [units: micromole/0.1 g liver]
20)	Amount of accumulated autooxidation = integral of (accumulated autooxidation + autooxidation) over time [units: micromole/0.1 g liver]
21)	Amount of accumulated hydroperoxides reduced by glutathione peroxidase = integral of (accumulated hydroperoxides reduced by glutathione peroxides + hydroperoxides reduced by glutathione peroxidase) over time [units: micromole/0.1 g liver]

Where: * is multiplication.

Numerical Values of BBPD Model Constants, Rates, and Factors Used in Simulations

Parameters used in BBPD model for simulation of lipid peroxidation in precision cut liver slices are listed in Table 2. The values were either adapted from the literature after Tappel et al. (1989), established experimentally, or simulated and optimized with SIMUSOLV software.

Table 2. Parameters for Precision Cut Liver Slices

Symbol	Numerical value	Description [Units]	Data source
Experimental setup:			
TSTOP	2.0	End of experiment and simulation [hr]	

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Table 2. Parameters for Precision Cut Liver Slices (cont'd)

Symbol	Numerical value	Description [Units]	Data source
IND1	5.0	Dose of chemical inducer 1 [micromole/0.1 g liver]	TR
IND1	7.83	Dose of chemical inducer 1 [micromole/0.1 g liver]	AM
BCKGD	0.0	TBARS in control samples [micromole/0.1 g liver]	
Chemical dependent:			
PTIND1	4.1	Potency of inducer 1 [1/micromole]	L
PTIND1	6.06	Potency of inducer 1 [1/micromole]	OB
PTIND1	6.90	Potency of inducer 1 [1/micromole]	OT
INDLF	0.1200	Inducer loss factor rate [1/hr]	L
INDLF	0.59	Inducer loss factor rate [1/hr]	OB
INDLF	0.275	Inducer loss factor rate [1/hr]	OT
ACTDGF	1.5	Activator degradation factor [1/micromole]	L
ACTDGF	3.75	Activator degradation factor [1/micromole]	OR
ACTDGF	3.43	Activator degradation factor [1/micromole]	OM
Animal dependent:			
PUF	7.0	Amount of polyunsaturated fat [micromole/0.1 g liver]	L
PXZLUF	12.0	Peroxidizability of L-PUFA factor [1/hr]	L
PXZHUF	24.0	Peroxidizability of H-PUFA factor [1/hr]	L
ANOX1	0.00500	Amount of Vit. E-type antioxidant [micromole/0.1 g liver]	L
ANOX1	0.00463	Amount of Vit. E-type antioxidant [micromole/0.1 g liver]	OR
ANOX1	0.00370	Amount of Vit. E-type antioxidant [micromole/0.1 g liver]	OM
ANOX2	0.0	Amount of non-Vit. E antioxidant [micromole/0.1 g liver]	

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Table 2. Parameters for Precision Cut Liver Slices (cont'd)

Symbol	Numerical value	Description [Units]	Data source
ANOX3	0.0	Amount of added antioxidant [micromole/0.1g liver]	
ACT1	0.0003	Amount of tissue activator 1 [micromole CYP/0.1 g liver]	L
GSH	0.6	Amount of reduced glutathione [micromole/0.1 g liver]	L
PXTTBA	0.1	Yield of TBARS from hydroperoxides [micromole/micromole]	L
LPUF	3.0	Amount of L-PUFA [micromole/0.1 g liver]	L
HPUF	4.0	Amount of H-PUFA [micromole/0.1 g liver]	L
EFANO1	1.0	Effectiveness of Vit. E coefficient	L
ANOXUF	2.0	Antioxidant use factor [1/micromole]	L
ACT2	0.0	Amount of activator 2 [micromole/0.1 g liver]	
ACACT1	1.0	Activity of activator 1 coefficient [micromole/micromole]	L
ACACT2	0.0	Activity of activator 2 coefficient [micromole/micromole]	
PXRATE	0.00029	Peroxidation rate coefficient [micromole/micromole]	L
AUTOXF	0.00012	Autooxidation factor rate [1/hr]	L
GPENZA	1.0	Activity of glutathione peroxidase [1/micromole]	L
GSH	0.6	Amount of reduced glutathione [micromole/0.1 g liver]	L
PXREDF	0.017	Hydroperoxide reduction factor rate [1/hr]	L
PHYSPX	0.0	Amount of physiological hydroperoxides [micromole/0.1 g liver]	

DATA SOURCE:
OB - optimized for treatment with BrCCl₃ (Tappel et al., 1989);
OT - optimized for treatment with TBOOH (this study);   L - from literature (Tappel et al., 1989);
TR - dose for rat liver (Tappel et al., 1989);   OM - optimized for mouse liver
AM - average dose for mouse liver (this study);   OR - optimized for rat liver
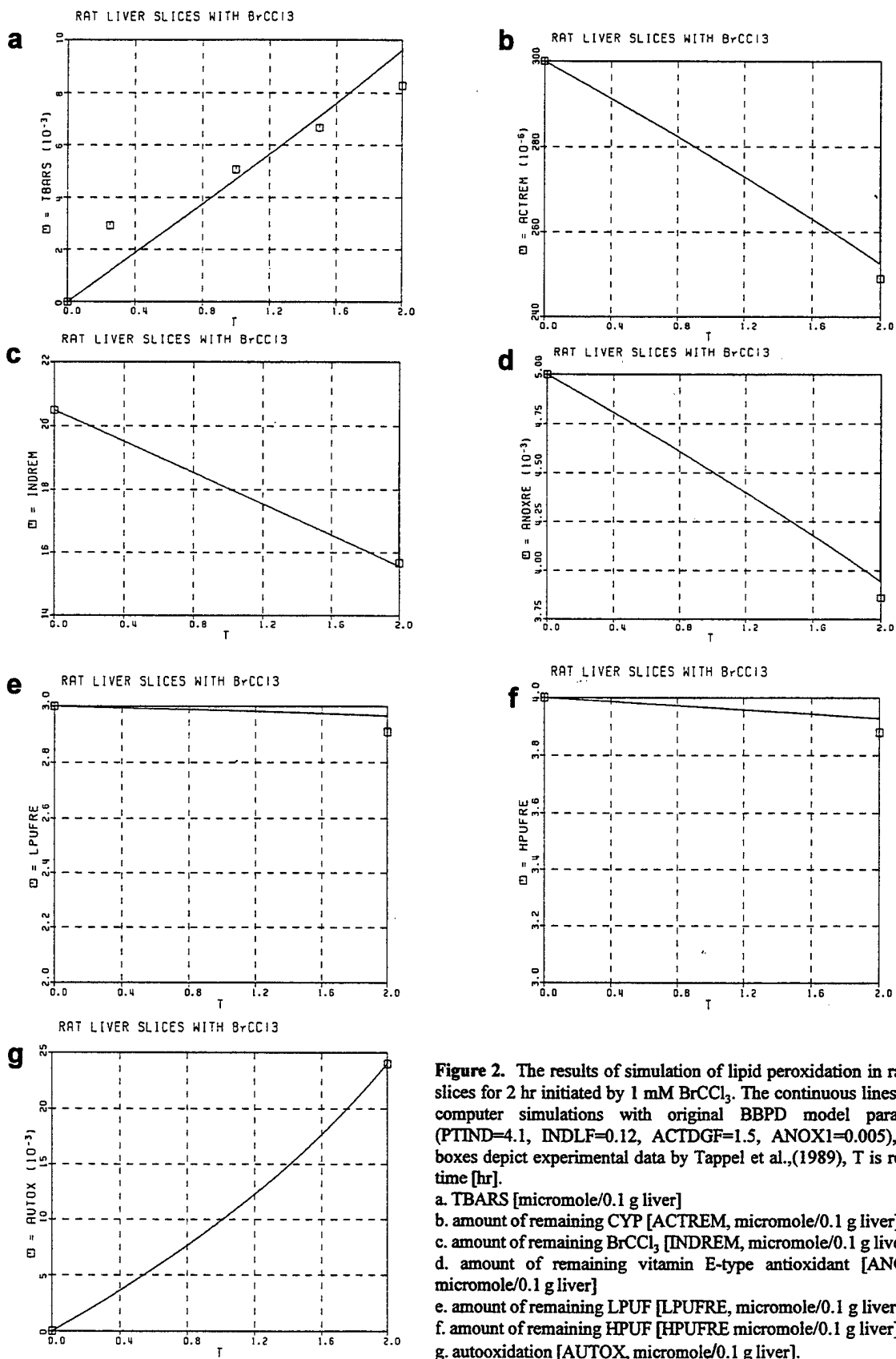
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Figure 2. The results of simulation of lipid peroxidation in rat liver slices for 2 hr initiated by 1 mM BrCCl$_3$. The continuous lines depict computer simulations with original BBPD model parameters (PTIND=4.1, INDLF=0.12, ACTDGF=1.5, ANOX1=0.005), small boxes depict experimental data by Tappel et al.,(1989), T is reaction time [hr].

a. TBARS [micromole/0.1 g liver]

b. amount of remaining CYP [ACTREM, micromole/0.1 g liver]

c. amount of remaining BrCCl$_3$ [INDREM, micromole/0.1 g liver]

d. amount of remaining vitamin E-type antioxidant [ANOXRE, micromole/0.1 g liver]

e. amount of remaining LPUF [LPUFRE, micromole/0.1 g liver]

f. amount of remaining HPUF [HPUFRE micromole/0.1 g liver]

g. autooxidation [AUTOX, micromole/0.1 g liver].

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Experimental Calibration of BBPD Model

Initially, we validated the BBPD model *versus* experimental data published by Tappel et al. (1989). The results of simulation of lipid peroxidation in rat liver slices for 2 hr, initiated by 1 mM bromotrichloromethane (BrCCl$_3$), are shown in Figure 2. Our model predicted in an almost linear fashion the amounts of TBARS (Figure 2 a), and gave close predictions of the Tappel et al. (1989) estimates of the amount of remaining CYP (ACTREM, Figure 2 b), the amount of remaining BrCCl$_3$ (INDREM, Figure 2 c), the amount of remaining vitamin E-type antioxidant (ANOXRE, Figure 2 d), the amounts of remaining LPUF (LPUFRE, Figure 2 e), and HPUF (HPUFRE, Figure 2 f), and the autooxidation (AUTOX, Figure 2 g).

After sensitivity analysis, the following BBPD model parameters were optimized with SIMUSOLV software: PTIND1, INDLF, ACTGF, and ANOX1. The optimization resulted in increased potency of inducer (PTIND=4.1 to 6.06), increased inducer loss factor (INDLF=0.12 to 0.59), increased CYP degradation factor (ACTDGF=1.5 to 3.75), and decreased content of vitamin E (ANOX1=0.005 to 0.00463) when compared to the original parameters published by Tappel et al. (1989). These changes significantly improved the fit to the TBARS data (Figure 3) without drastically changing the accuracy of predictions of the Tappel et al. (1989) estimates (ACTREM, INDREM, ANOXRE, LPUFRE, HPUFRE, AUTOX).

Without changing the animal-dependent BBPD model parameters, we ran a simulation of lipid peroxidation in rat liver slices for 2 hr, initiated by 1 mM TBOOH. The results are shown in Figure 4 along with data published by Fraga et al. (1988). Because the experimental results were originally published without subtracting the background amount of TBARS produced at time=0, the BBPD model parameter BCKGD=0.0014 was set for simulation (Figure 4). For further simulations the background amounts of TBARS at time=0 were routinely subtracted from the data. From these simulations the chemical-dependent parameters were optimized as follows: PTIND1=6.9, INDLF=0.275, ACTDGF=3.43.

Without changing these chemical-dependent BBPD model parameters, we ran a simulation of lipid peroxidation in mouse liver slices for 2 hr, initiated by 1 mM TBOOH. The results are shown in Figure 5 along with data from our laboratory. The amount of TBARS at time=0 was subtracted from the data. From this simulation, we optimized the animal-dependent parameter ANOX1=0.0037. This difference (ANOX1=0.0037 *vs* 0.00463), corresponds to the known lower concentration of vitamin E in mouse liver than in the rat (Sugimoto et al., 1991).
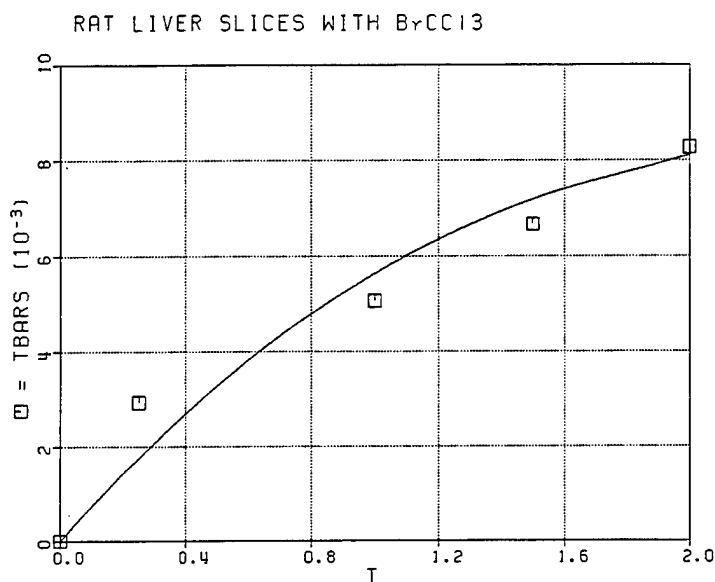

Figure 3. The results of simulation of lipid peroxidation in rat liver slices for 2 hr, initiated by 1 mM BrCCl$_3$. The continuous lines depict computer simulations with BBPD model parameters optimized by SMUSOLV software (PTIND=6.06, INDLF=0.59, ACTDGF=3.75, ANOX1=0.00463), small boxes depict experimental data by Tappel et al. (1989), T is reaction time [hr].
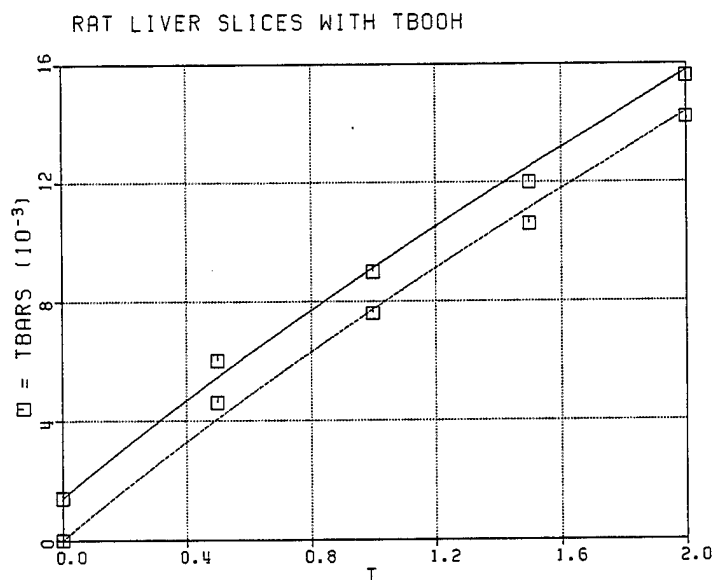
RAT LIVER SLICES WITH TBOOH

Figure 4.   The results of simulation of lipid peroxidation in rat liver slices for 2 hr, initiated by 1 mM TBOOH. The continuous lines depict computer simulations with BBPD model parameters optimized by SIMUSOLV software (PTIND1=6.9, INDLF=0.275, ACTDGF=3.43; ANOX1=0.00463 was the same as in Figure 3), small boxes depict experimental data by Fraga et al. (1988) with BCKGD=0 (upper curve) and BCKGD=0.0014 (lower curve), T is reaction time [hr].

Table 3. Sensitivity Analysis of BBPD Model

Peroxidation parameter	Percent change of output (TBARS) in response to 10% increase in input parameter
PUF	- 0.0015
PXZLUF	+ 2.2500
PXZHUF	+ 5.8300
ANOX1	-11.7340
ACT1	+ 7.9950
IND1	+ 7.9950
GSH	- 0.9790
PXTTBA	+10.0070
LPUF	+ 4.8230
HPUF	+ 9.6200
ANOXUF	+ 7.6860
ACTDGF	- 7.1670

Table 3. Sensitivity Analysis of BBPD Model (cont'd)·

Peroxidation parameter	Percent change of output (TBARS) in response to 10% increase in input parameter
PTIND1	+ 7.9950
INDLF	- 2.2160
PXRATE	+ 7.9950
AUTOXF	+ 0.8390
GPENZA	- 0.9400
PXREDF	+ 9.2720

Peroxidation parameters were increased by 10% above the defaulted values used in simulation shown in Figure 5; TBARS values after 2 hr were the responding output variable; the values are expressed as percent (+ or -) of TBARS value at 2 hr in Figure 5 (0.02585 micromole/0.1 g liver).

MOUSE LIVER SLICES WITH TBOOH

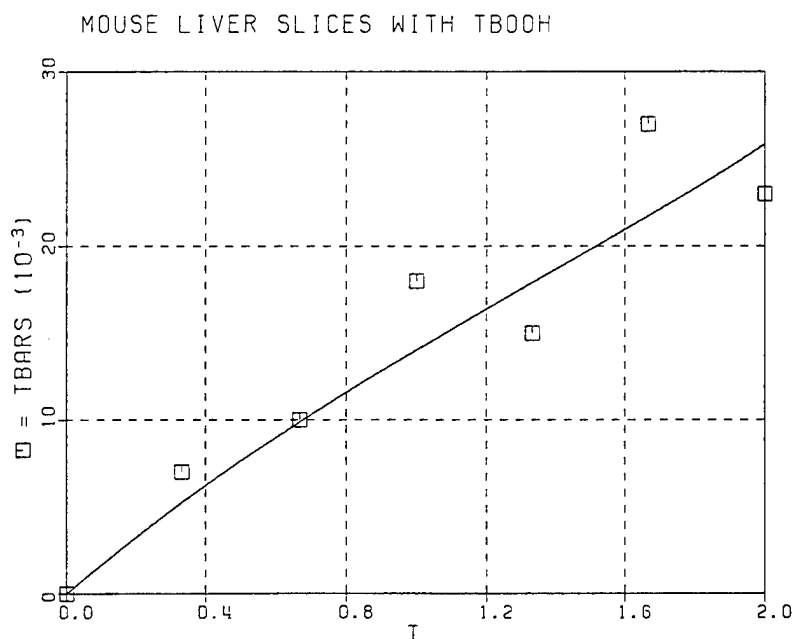**Figure 5.** The results of simulation of lipid peroxidation in mouse liver slices for 2 hr, initiated by 1 mM TBOOH. The continuous lines depict computer simulations with BBPD model parameters optimized by SIMUSOLV software (ANOX1=0.0037; the remaining parameters were the same as in Figure 4: PTIND1=6.9, INDLF=0.275, ACTDGF=3.43), amount of TBARS at time=0 was subtracted from the data, T is reaction time [hr].

SECTION 4

DISCUSSION

In the recent literature, there are published several mathematical approaches that may be useful in describing the kinetics of free radical reactions leading to oxidative damage of living cells and tissues (e.g., Tappel, et al., 1989; Babbs and Steiner, 1990; Suzuki and Ford, 1994; Vroegop et al., 1995). However, only a few have been actually calibrated or somehow validated in the biological material (Tappel et al., 1989; Vroegop et al., 1995). In the case of lipid peroxidation (or TBARS production) simulation as an end point of the oxidative stress in cells, it seems especially important to consider the biological specificity of the cellular system, its antioxidant capacity, and its ability to degrade the free radical activator as well as the ability to regenerate reductive cofactors (e.g., vitamin E, or GSH). Moreover, once initiated in certain tissue preparations, the lipid peroxidation may propagate non-enzymatically due to "autooxidation" in the presence of catalytic amounts of transition metals (Byczkowski and Kulkarni, 1989). In the living organisms, *in vivo*, the chemically initiated oxidative stress may be further complicated by compartmentation, disposition, and metabolism of the chemical inducer as well as by sometimes opposite responses of tissues (e.g., proliferation, apoptosis or necrosis; Byczkowski and Channel, 1995). Most of these complicating factors were successfully incorporated in the iterative model by Tappel et al. (1989). Moreover, their model was calibrated experimentally with rat liver slices, and thereafter, their mathematical approach was successfully used to describe the requirement for antioxidants *in vivo* (Tappel, 1992). Thus, from the review of existing mathematical approaches and models, the iterative model of Tappel et al. (1989) seemed to be the most promising for development of a biologically based pharmacodynamic model of lipid peroxidation, suitable for interlinking with PBPK (Byczkowski et al., 1995).

In this developed model, several restrictions have been imposed. From the sensitivity analysis (Table 3) it was clear that the level of vitamin E-type antioxidants was the most critical parameter. Under physiological conditions, the changes of antioxidant concentrations in the tissue are limited due to regeneration and supplementation with diet (Leibovitz et al., 1990; Hu et al., 1989; Pellett et al., 1994). However, *in vitro*, vitamin E-type antioxidants may be easily depleted by prolonged incubation, drastic oxidative stress, and high concentration of free radical-generating xenobiotics. In such circumstances, a rapid increase in lipid peroxidation was accompanied by decreased viability and necrosis (results notshown). Once the antioxidant level drops to zero, the tissue dies. To keep our experimental calibration within conditions relevant to the intoxication scenarios that may occur *in vivo*, we have limited the

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incubation times of liver slices with xenobiotic to a maximum of two hours, and have limited concentrations of xenobiotic to the range in which lipid peroxidation was not yet accompanied by a decrease of tissue viability or necrosis. These precautions have assured us that the model is being calibrated with live tissue, and not with a necrotic or dying one. Accordingly, in the BBPD model, a special logical "IF" statement preventing the amount of antioxidants from dropping to zero was set in the "PROCEDURAL" block. Similar logical "IF" statements were used in the BBPD model to prevent several variables from assuming physiologically impossible or negative values (APPENDIX).

Since in response to the 10% input constants change the output was changing by 10% or less (Table 3), it seems that the BBPD model was stable. A single exception from this was the amount of Vitamin E-type antioxidant (ANOX1) parameter, reflecting the high sensitivity of liver and other biosystems to depletion of natural antioxidants. The BBPD model describes adequately the balance between initiation of lipid peroxidation and protection against it. The natural negative feedback by inhibition of CYP and positive feedback by depletion of antioxidants and stimulation of autooxidation are incorporated in the BBPD model.

It is important to emphasize that the developed BBPD model is not just another exercise in curve fitting, but it kept track in a realistic way of the concentrations of reactants, updated as time passed, and integrated them as molar amounts of mass transferred from one product of lipid peroxidation to another over time.

It seems that this model may be applied in the future modifications for comparison of a relative intrinsic hepatotoxicity related to lipid peroxidation in different species (e.g., mouse, rat, human) *in vivo*, based on information gained from the *in vitro* experiments. It may be also applied for comparison of a relative peroxidative potency of different chlorinated hydrocarbons (e.g., CCl_4, $CHCl_3$, $BrCCl_3$, TCE) with other initiators of lipid peroxidation, such as tert-butyl hydroperoxide. It may be also used for simulation of the possible prooxidant effects of diet (polyunsaturated fat, antioxidants, etc.), as well as occupational and environmental contaminants.

SECTION 5

CONCLUSIONS

The constructed BBPD model allowed us to simulate successfully the lipid peroxidation process in precision cut mouse liver slices initiated *in vitro* by a known tumor promoter, TBOOH.

The BBPD model may be applied to simulate lipid peroxidation initiated by different chemicals in liver slices from different animal species.

Copies of the computer program may be obtained for non-commercial use by sending E-mail request to the following Internet address:

JBYCZKOWSKI%RAVEN@EAGLE.AL.WPAFB.AF.MIL

SECTION 6

REFERENCES

Allevi, P., M. Anastasia, F. Cajone, P. Ciuffreda, and A.M. Sanvito. 1995. Structural requirements of aldehydes produced in LPO for the activation of heat-shock genes in HeLa cells. Free Radical Biology and Medicine 18:107-116.

Babbs, C.F., and M.G. Steiner. 1990. Simulation of free radical reactions in biology and medicine: a new two-compartment kinetic model of intracellular lipid peroxidation. Free Radical Biology and Medicine 8:471-485.

Brambilla, G., A. Martelli, and U.M. Marinari. 1989. Is lipid peroxidation associated with DNA damage? Mutation Research 214:123-127.

Brendel, K.L, A.J. Gandolfi, C.L. Krumdieck, and P.F. Smith. 1987. Tissue slicing and culture revisited. Trends in Pharmacological Sciences 8:12-15.

Brendel, K., R.L. Fisher, C.L. Krumideck, and A.J. Gandolfi. 1993. Precision-cut rat liver slices in dynamic organ culture for structure-toxicity studies. In: Methods in Toxicology. Vol. 1A, pp. 222 - 226. Academic Press, New York, NY.

Byczkowski, J.Z., and S.R. Channel. 1995. Chemically-induced oxidative stress and tumorigenesis: effects on signal transduction and cell proliferation. Toxic Substance Mechanisms In press.

Byczkowski, J.Z., and A.P. Kulkarni. 1989. NADPH-dependent drug redox cycling and lipid peroxidation in microsomes from human term placenta. Int. J. Biochem. 21:183-190.

Byczkowski, J.Z., T. Pravecek, and S. Channel. 1995. Computer simulations of lipid peroxidation in precision cut liver slices and experimental calibration of the mathematical model. Toxicologist 15:281(1504).

Chaudhary, A.K., M. Nokubo, G.R. Reddy, S.N. Yeola, J.D. Morrow, I.A. Blair, and L.J. Marnett. 1994. Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science 265:1580-1582.

De Groot, H., and A. Littauer. 1989. Hypoxia, reactive oxygen and cell injury. Free Radical Biology and Med. 6:541-551.

Perchellet, J.P., and E.M. Perchellet. 1989. Antioxidants and multistage carcinogenesis in mouse skin. Free Radical Biology and Medicine 7:377-408.

Sipes, I.G., R.L. Fisher, P.F. Smith, E.R. Stine, A.J. Gandolfi, K. Brendel. 1987. A dynamic liver culture system: a tool for studying chemical biotransformation and toxicity. Arch. Toxicol. Suppl. 11:20-33.

Slater, T.F. 1984. Free-radical mechanisms in tissue injury. Biochemical Journal 222:1-15.

Sugimoto, H., S. Matsuzaki, K. Hamana, S. Yamada, and S. Kobayashi. 1991. alpha-Tocopherol and superoxide dismutase suppress and diethyldithiocarbamate and phorone enhance the lipopolysaccharide-induced increase in N1-acetylspermidine concentrations in mouse liver. Circulatory Shock 33:171-177.

Suzuki, Y.J., and G.D. Ford. 1994. Mathematical model supporting the superoxide theory of oxygen toxicity. Free Radical Biology and Medicine 16:63-72.

Tappel, A. 1992. Combinations of vitamin E and other antioxygenic nutrients in protection of tissues. In: Vitamin E in Health and Disease. Edited by L. Packer & J. Fuchs. pp. 313-325. Marcel Dekker, NY.

Tappel, A.L., A.A. Tappel, and C.G. Fraga. 1989. Application of simulation modeling to lipid peroxidation process. Free Radical Biology and Medicine 7:361-368.

Van Der Vliet, A., and A. Bast. 1992. Effect of oxidative stress on receptors and signal transmission. Chemico - Biological Interactions 85:95-116.

Von Ruecker, A.A., B.G. Han-Jeon, M. Wild, and F. Bidlingmaier. 1989. Protein kinase C involvement in lipid peroxidation and cell membrane damage induced by oxygen-based radicals in hepatocytes. Biochemical and Biophysical Research Communications 163:836-842.

Vroegop, S.M., D.E. Decker, and S.E. Buxser. 1995. Localization of damage induced by reactive oxygen species in cultured cells. Free Radical Biology and Medicine 18:141-151.

Yang, S.H., and M.E. Andersen. 1994. Pharmacokinetics. In: Introduction to Biochemical Toxicology (Hodgson, E., P.E. Levi, Eds), Chapter 3,pp.49-73, Appleton & Lange,Norwalk, CT.

Flohe, L., H. Giertz, and R. Beckman. 1985. Free radical scavengers as anti-inflammatory drugs? In: Handbook of Inflammation (Bonta, I.L., M.A. Bray, M.J. Parnham, Eds), Vol. 5, Chapter 13, pp. 255-281, Elsevier Science Amsterdam.

Fraga, C.G., B.E. Laibovitz, and A.L. Tappel. 1988. Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. Free Radical Biology and Medicine 4:155-161.

Gardner, H.W. 1989. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radical Biology and Medicine 7:65-86.

Mitchell and Gauthier Associates, Inc. 1993. Advanced Continuous Simulation Language (ACSL). Reference Manual. Concord, MA.

Hu, M.-L., E.N. Frankel, B.E. Leibovitz, and A.L. Tappel. 1989. Journal of Nutrition 119:1574-1589.

Janero, D.R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine 9:515-540.

Kappus, H., and H. Sies. 1981. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. Experientia 37:1233-1358.

Krumdieck, C.L, J.E. Dos Santos, and K.J. Ho. 1980. A new instrument for the rapid preparation of tissue slices. Analytical Biochemistry 104:118-123.

Leibovitz, B.E., M.-L. Hu, and A.L. Tappel. 1990. Lipid peroxidation in rat tissue slices: effect of dietary vitamin E, corn oil-lard and menhaden oil. Lipids 25:125-129.

Mitchell and Gauthier Associates, 1993. Advanced Continuous Simulation Language (ACSL) Reference Manual. Edition 10.1. MGA, Inc. Concord, MA.

Nicotera, P., J.M. Dypbukt, A.D. Rossi, L. Manzo, and S. Orrenius. 1992. Thiol modification and cell signalling in chemical toxicity. Toxicology Letters 64/65:563-567.

Pellett, L.J., H.J. Andersen, H. Chen, and A.L. Tappel. 1994. β-carotene alters vitamin E protection against heme protein oxidation and lipid peroxidation in chicken liver slices. Journal of Nutrition Biochemistry 5:1-7.

Source Codes of BBPD Model Written in ACSL. The *.CSL and *.CMD files should be executed under SIMUSOLV

*.CSL File

```
PROGRAM: LIPID PEROXIDATION
'THE PURPOSE OF THIS PROGRAM IS TO SIMULATE PRODUCTION OF TBARS'
'FROM PUFA IN LIVER SLICES in vitro.                           '


INITIAL


'DEFAULT PEROXIDATION PARAMETERS FOR LIVER, ESTIMATED FROM      '
'Tappel et al. (1989) [per 0.1 g of liver]                     '


CONSTANT    PUF   = 7.0        $'[microMol/0.1 g liver]        '
CONSTANT    PXZLUF= 12.0       $'peroxidizability of PUFA L[1/h]'
CONSTANT    PXZHUF= 24.0       $'peroxidizability of PUFA H[1/h]'
CONSTANT    ANOX1 = 0.005      $'Vit.E antiox[microMol/0.1g liv]'
CONSTANT    ACT1  = 0.0003     $'activator1[mcM cytP450/0.1gliv]'
CONSTANT    IND1  = 5.0        $'inducer 1 [mcM BrCCl3/0.1g liv]'
CONSTANT    GSH   = 0.6        $'glutathione [mcroM/0.1 g liver]'
CONSTANT    PXTTBA= 0.1        $'yield ofTBARS/Mol hydroperoxide'
CONSTANT    LPUF  = 3.         $'LA-derivative PUFA[mcMol/0.1 g]'
CONSTANT    HPUF  = 4.         $'HA-derivative PUFA[mcMol/0.1 g]'
CONSTANT    ANOX2 = 0.         $'non-Vit.E antiox[mcM/0.1 g liv]'
CONSTANT    ANOX3 = 0.         $'added antioxidant[mcM/0.1g liv]'
CONSTANT    EFANO1 = 1.        $'effectiveness of Vit.E         '
CONSTANT    EFANO2 = 0.        $'effectiveness of non-Vit.E a-o '
CONSTANT    EFANO3 = 0.        $'effectiveness of added a-o     '
CONSTANT    ANOXUF = 2.        $'antioxidant use factor [/mcmol]'
CONSTANT    ACT2   = 0.        $'activator 2 [mcM/0.1 g liver]  '
CONSTANT    ACACT1 = 1.        $'activity of activator 1        '
CONSTANT    ACACT2 = 0.        $'activity of activator 2        '
CONSTANT    ACTDGF = 1.5       $'activator degradation fctr /mcm'
```

```
CONSTANT    IND2   = 0.        $'inducer2[mcM chemical/0.1g liv]'
CONSTANT    PTIND1 = 4.1       $'potency of inducer 1 [1/mcmol] '
CONSTANT    PTIND2 = 0.        $'potency of inducer 2 [1/mcmol] '
CONSTANT    INDLF  = 0.1200    $'inducer loss factor [1/h]      '
CONSTANT    PXRATE = 0.00029   $'peroxidation rate [mcm/mcm]    '
CONSTANT    AUTOXF = 0.00012   $'autooxidation factor [1/h]     '
CONSTANT    GPENZA = 1.        $'glutathione peroxidase[/mcM]   '
CONSTANT    PXREDF = 0.017     $'hydroperoxide reduction fctr /h'
CONSTANT    PHYSPX = 0.        $'physiological levels of Hperox '
CONSTANT    ANREG  = 0.        $'antioxidant regenerated in vivo'
CONSTANT    BCKGD  = 0.0       $'TBARS in control [mcm/0.1 g]   '
CONSTANT    TSTOP  = 2.        $'end of simulation [h]          '


'TIMING COMMANDS                                                 '
CONSTANT    POINTS = 500.
CINT=TSTOP/POINTS             $'communication interval          '


'                    INITIALIZATION                              '
'RESETS INITIAL CONDITIONS BEFORE PEROXIDATION                   '
CONSTANT ACTLOS=0.            $'activator loss                  '
CONSTANT AUTOX =0.            $'autooxidation                   '
CONSTANT PXREDG=0.            $'Hperoxides red. by GSH peroxidase'
CONSTANT PXLUF =0.00199       $'L-Hydroperoxides formed         '
CONSTANT PXHUF =0.0053        $'H-Hydroperoxides formed         '
CONSTANT PXREM =0.            $'accumulated remaining Hperoxides '
CONSTANT TPX   =0.            $'accumlted total Hperoxides formed'
CONSTANT AUTOXA=0.            $'no autooxidation                '
CONSTANT PXREDA=0.            $'no reduced Hperoxides           '
CONSTANT PXHUFA=0.            $'no Hperoxides from H-PUFA        '
CONSTANT PXLUFA=0.            $'no Hperoxides from L-PUFA        '
CONSTANT ILR   =0.            $'rate of inducer loss = 0.       '
CONSTANT INDLOS=0.            $'no inducer lost                 '


END                          $'End of Initial                  '


DYNAMIC
```

```
        ALGORITHM IALG = 2            $'Gear stiff method                          '


DERIVATIVE


PROCEDURAL


IF (LPUFRE.LE.0) LPUFRE = 0.
IF (HPUFRE.LE.0) HPUFRE = 0.
IF (ACTLOS.GE.ACTEF)  ACTLOS = ACTEF
IF (ANOXRE.LE.1.e-10) ANOXRE = 1.e-10
IF (GSHREM.LE.0) GSHREM = 0.
IF (INDLOS.GE.INDEF)  INDLOS = INDEF
IF (PXREDA.GE.TPX) PXREDA = TPX


END     $'End of procedural                                                        '


   '    Amounts of remaining polyunsaturated fatty acids                          '
 LPUFRE = LPUF - PXLUFA - AUTOXA/2
 HPUFRE = HPUF - PXHUFA - AUTOXA/2


   '    Amount of effective activator                                             '
 ACTEF =  ACT1*ACACT1 + ACT2*ACACT2


   '    Activator loss                                                            '
 ACTLOS = ACTEF*ACTDGF*TPX


   '    Amount of remaining activator                                             '
 ACTREM = ACTEF - ACTLOS


   '    Amount of effective inducer                                               '
 INDEF = IND1*PTIND1 + IND2*PTIND2


   '    Amount of remaining inducer                                               '
 INDREM = INDEF - INDLOS


   '    Inducer loss rate                                                         '
```

```
ILR = INDEF*INDLF


'    Amount of activated inducer                                  '
ACTIND = INDREM*ACTREM


'    Amount of effective antioxidant                              '
ANOXEF = ANOX1*EFANO1 + ANOX2*EFANO2 + ANOX3*EFANO3


'    Amount of remaining antioxidant                             '
ANOXRE = ANOXEF - ANOXEF*TPX*ANOXUF + ANREG


'    Rate of hydroperoxides formation by action of activated     '
'    inducer on PUFA                                             '
PXLUF = LPUFRE*PXZLUF*ACTIND*PXRATE/ANOXRE
PXHUF = HPUFRE*PXZHUF*ACTIND*PXRATE/ANOXRE


'    Rate of autooxidation                                       '
AUTOX = (LPUFRE + HPUFRE)*AUTOXF*TPX/ANOXRE


'    Amount of accumulated total hydroperoxides formed           '
TPX = AUTOXA + PXLUFA + PXHUFA + PHYSPX


'    Amount of remaining glutathione                             '
GSHREM = GSH - PXREDA


'    Rate of hydroperoxides reduced by glutathione peroxidase    '
PXREDG = PXREM*GPENZA*GSHREM*PXREDF


'    Amount of accumulated remaining hydroperoxides              '
PXREM = TPX - PXREDA


'    Amount of TBARS from accumulated remaining hydroperoxides   '
TBARS = PXREM*PXTTBA + BCKGD


'    Inducer loss                                                '
INDLOS = INTEG(ILR,0.)
```

```
'    Amount of accumulated hydroperoxides formed by action of    '
'    activated inducer on PUFA                                    '
PXLUFA = INTEG(PXLUF, 0.)


PXHUFA = INTEG(PXHUF, 0.)


'    Amount of accumulated autooxidation                          '
AUTOXA = INTEG(AUTOX, 0.)


'    Amount of accumulated hydroperoxides reduced by glutathione'
'    peroxidase                                                   '
PXREDA = INTEG(PXREDG, 0.)


 TERMT(T.GE.TSTOP)              $'Termination at TSTOP            '


END                            $'End of derivative               '
END                            $'End of dynamic                  '
'^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^'
TERMINAL                       $'Resets parameters to initial values'
ACTREM = ACTEF
INDREM = INDEF
ANOXRE = ANOXEF
END                            $'End of terminal                 '
'^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^^'
END                            $'End of program                  '



*.CMD File


PROCED FIG2
'Fig. 2 '
SET TITLE='RAT LIVER SLICES WITH BrCCl3'
SET TSTOP=2., NRWITG=.F.,
PREPAR T,TBARS,ACTLOS,INDREM,ANOXRE,AUTOX,PXREDA,AUTOXA,HPUF
PREPAR PXHUFA,PXLUFA,PXREM,PXREDG,GSHREM,TPX,PXHUF,PXLUF,LPUF
```

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```
2.    0.00827


END      $'END OF DATA'
START
PLOT TBARS
END      $ END OF FILE'


PROCED FIG4
'Fig. 4 '
SET TITLE='RAT LIVER SLICES WITH TBOOH'
SET TSTOP=2.,
PREPAR T,TBARS,ACTLOS,INDREM,ANOXRE,AUTOX,PXREDA,AUTOXA,HPUF
PREPAR PXHUFA,PXLUFA,PXREM,PXREDG,GSHREM,TPX,PXHUF,PXLUF,LPUF
PREPAR ANOXEF,ACTIND,ACTREM,INDEF,ACTEF,HPUFRE,LPUFRE,HPUF,INDLOS
SET PTIND1=6.9,IND1=5.,INDLF=0.275,ACTDGF=3.43,ANOX1=0.00463
SET NRWITG=.T., BCKGD=0.0014


START
SET BCKGD=0.


'[h]    [mcm/0.1g]'
DATA


T        TBARS
0.       0.0014     INITIAL
0.5      0.0060
1.       0.0090
1.5      0.0120
2.       0.0156
0.       0.0          INITIAL
0.5      0.0046
1.       0.0076
1.5      0.0106
2.       0.0142


END      $'END OF DATA'
```

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```
PREPAR ANOXEF,ACTIND,ACTREM,INDEF,ACTEF,HPUFRE,LPUFRE,HPUF,INDLOS
SET PTIND1=4.1,ACTDGF=1.5,INDLF=0.12,ANOX1=0.005,IND1=5.


'       a.       c.      b.       d.      e.      f.      g.'
'[h] [micromole/0.1 g liver]'
DATA


T    TBARS     INDREM   ACTREM   ANOXRE LPUFRE HPUFRE  AUTOX
0.   0.        20.5     0.0003   0.005  3.0    4.0      0.   INITIAL
0.25 0.00293   .        .        .      .      .        .
1.   0.00507   .        .        .      .      .        .
1.5  0.00667   .        .        .      .      .        .
2.   0.00827 15.68      0.000249 0.00386 2.91 3.88      0.024


END       $'END OF DATA'
START
PLOT TBARS
END       $ END OF FILE'


PROCED FIG3
'Fig. 3 '
SET TITLE='RAT LIVER SLICES WITH BrCCl3'
SET TSTOP=2., NRWITG=.F.,
PREPAR T,TBARS,ACTLOS,INDREM,ANOXRE,AUTOX,PXREDA,AUTOXA,HPUF
PREPAR PXHUFA,PXLUFA,PXREM,PXREDG,GSHREM,TPX,PXHUF,PXLUF,LPUF
PREPAR ANOXEF,ACTIND,ACTREM,INDEF,ACTEF,HPUFRE,LPUFRE,HPUF,INDLOS
SET PTIND1=6.06,ACTDGF=3.75,INDLF=0.59,ANOX1=0.00463,IND1=5.


'[h]    [mcm/0.1g]'
DATA


T    TBARS
0.    0.
0.25 0.00293
1.    0.00507
1.5   0.00667
```

```
START

PLOT TBARS

END        $'END OF FILE'


PROCED FIG5

'Fig. 5 '

SET TITLE= 'MOUSE LIVER SLICES WITH TBOOH'

SET TSTOP=2., NRWITG=.F., BCKGD=0.

PREPAR T,TBARS,ACTLOS,INDREM,ANOXRE,AUTOX,PXREDA,AUTOXA,HPUF

PREPAR PXHUFA,PXLUFA,PXREM,PXREDG,GSHREM,TPX,PXHUF,PXLUF,LPUF

PREPAR ANOXEF,ACTIND,ACTREM,INDEF,ACTEF,HPUFRE,LPUFRE,HPUF,INDLOS

SET PTIND1=6.9,ANOX1=0.0037,INDLF=0.275,ACTDGF=3.43,IND1=7.83


'[h]    [mcm/0.1g]   [mcm/0.1g]'

DATA


T          TBARS       IND1

0.         0.0         9.1

0.333      0.007       8.7

0.667      0.010       7.7

1.0        0.018       7.6

1.333      0.015       7.8

1.667      0.027       7.6

2.0        0.023       7.6


END        $'END OF DATA'

START

PLOT TBARS

END        $'END OF FILE'
```